CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 20-903

PHARMACOLOGY REVIEW(S)

PHARMACOLOGIST'S REVIEW

NDA: 20-903 Original

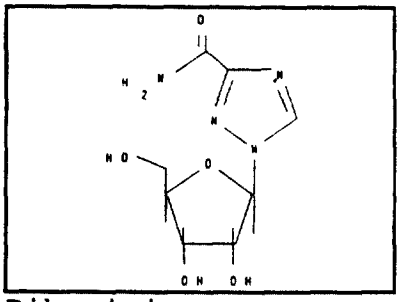
Date Submitted: 5 Dec. 1997
Date Assigned: 5 Dec. 1997
Date Review Completed: 8 May 1998
Reviewer: David E. Morse, Ph.D.
HFD-530: Div. of Antiviral Drug Products

SPONSOR: Schering Corporation
Galloping Hill Road
Kenilworth, NJ 07033 (908) 298-4000

DRUG: Ribavirin (Rebetol® and Virazole®)
1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide

and, Interferon alpha-2b (Intron A®; PLA No. 994)

STRUCTURE:



Ribavirin

EMPIRICAL FORMULA: $C_8H_{12}N_4O_5$
MOLECULAR WEIGHT: 244.21
MELTING POINT: 166-168°C in aq. ETOH
174-176°C in ETOH
SOLUBILITY: 142 mg/ml (H_2O, at 25°C)

FORMULATION: Capsules, 200 mg each

INDICATION: Treatment of Hepatitis C infection

RELATED IND(s)/NDA(s)/PLA(s):

NDAs 18-266 (ICN); 18-859 (ICN); 19-705 (U.S. Army); 20-206 (ICN);

PLAs 994 (Schering Corp.)

DEFINITIONS:[1]

INTRODUCTION

The sponsor has submitted a new NDA for the use of ribavirin capsules (REBETOL®; 1-1.2 g/day; PO) in combination with interferon alpha-2b (INTRON A®; 3 MIU TIW; SC Inj.) for the treatment of chronic active Hepatitis type C infection in patients who have relapsed following a previous course of interferon monotherapy. The proposed duration of combination drug administration in the treatment of Hepatitis type C infection is 6 months. Interferon alpha (INTRON A®, and related interferons: ROFERON®, WELLFERON®, INFERGEN®) has been previously approved for use at the proposed dose and route of administration for the treatment of Hepatitis type C infection. Since no change in the indication or use of interferon alpha has been proposed in this NDA, the reader is referred to the PLA review (No. 994) for a comprehensive review of the toxicology of INTRON A® brand of interferon alpha-2b. In contrast, ribavirin is currently only approved as an aerosol product (VIRAZOLE®; NDA 18-859) for use in the treatment of Respiratory Syncytial Virus (RSV) infection in the pediatric population. No oral ribavirin containing product is currently available for use in the US. Therefore, this NDA review will focus primarily on the repeat-dose toxicology, reproductive

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[1] Virazole^R- ICN Pharm, Inc., Costa Mesa, CA

toxicology, genotoxicity and carcinogenicity, pharmacokinetic and ADME studies related to the use of ribavirin.

The following sections of this document contain reviews and/or summaries of the toxicology studies contained in this or related submissions. A discussion of additional toxicology requirements regarding the long-term human use of ribavirin is contained in the summary section of this document. Proposed product labeling for the combined use product (INTRON a and REBETOL) is contained in the last appendix to this document.

BACKGROUND

Interferon alfa-2b is obtained from bacterial fermentation of a genetically engineered strain of Escherichia coli containing the human interferon gene. Interferons are a class of related glycoproteins (MW range of 15-28 $x10^3$) which are secreted by multiple body tissues in response to viral infection and various other inducers. Interferons bind to specific cell surface receptors and initiate intracellular changes which cause changes in cell proliferation, phagocytic and cytotoxic activity of lymphocytes, and inhibit viral replication. Interferon alfa-2b is currently approved for IV, SC or IM administration in the treatment of hepatitis B and C, NANB/C hepatitis, hairy cell leukemia, AIDS-related Kaposi's sarcoma, and external condylomata acuminata.

Ribavirin is a purine nucleoside analogue which has shown in vitro inhibitory activity against multiple DNA and RNA viruses. A proposed mechanism for the antiviral activity of ribavirin is through changes in the metabolism of guanosine and/or xanthosine. Ribavirin is currently approved only for the treatment of severe lower respiratory tract infections due to respiratory syncytial virus (RSV) in children. RSV is a member of the Paramyxo virus family, along with measles, mumps, para-influenza, and Newcastle disease viruses. All of the viruses in this family are RNA viruses, as is the causative agent in hepatitis C.

Ribavirin is approved only for aerosol administration by face mask, hood, tent or mechanical ventilator in the treatment of severe lower respiratory tract infections due to RSV in pediatric patients. There is no oral or intravenous formulation of ribavirin which is approved for use in the U.S. at this time.

NON-CLINICAL TOXICOLOGY

The following sections contain summaries of the major toxicology and pharmacokinetic data submitted as part of the NDA. The individual study reviews the proposed package insert for the combination drug preparation are included in appendices A-E of this document.

Ribavirin: Summary of General Toxicology Findings

Pre-clinical toxicology data indicate that ribavirin induces a significant degree of anemia (due to direct hemolytic effects and suppression of the bone marrow), reticulocytosis, and lymphoid atrophy following high dose, acute administration or low dose, repeat administration. The anemia is generally reversed within a few weeks following the cessation of ribavirin administration. Study data suggest that accumulation of ribavirin occurs in body tissues during repeat dosing procedures, but that it generally stabilizes 1-3 weeks following the start of dosing (although more gradual accumulation may continue up to 6 months of dosing).

The results of multiple studies suggest that the administration of ribavirin may be associated with reductions in serum protein, albumin and ALT levels among dogs dosed at 20 mg/kg/day and among rats dosed at 160 mg/kg/day (smaller effects sometimes evident among animals dosed at lower levels; estimated human equivalent doses of 10 and 23 mg/kg/day [based on body surface area conversion]). Histologic changes evident in either the liver or kidneys were not consistent with these changes. The pattern of the data suggests that the changes in serum protein, albumin and ALT levels may have resulted from a ribavirin induced inhibition of the synthetic capacity of the liver. Further, the results of these studies (rat and dog) suggest that ribavirin, when given at relatively high doses, has significant adverse effects on rapidly proliferating tissues (lymphoid, mucosa and spleen) and/or those tissues with high cellular metabolism (heart, liver and secretory cells of the intestinal mucosa).

Ribavirin: Summary of Carcinogenicity Study Findings

The in vivo carcinogenicity studies performed with ribavirin were inadequately designed (drug doses too low), were not conducted in accordance with the study protocols or were incomplete, and were inadequately reported/documented. Thus, the results of the 2 oral gavage oncogenicity studies in the mouse and rat (18-24 months; doses of 20-75 and 10-40 mg/kg/day, respectively [estimated human equivalent doses of 1.67-6.25 and 1.43-5.71 mg/kg/day, based on body surface area adjustment for the adult]; approximately 1.3x and 0.2x the human systemic exposure to ribavirin [based on 24 hour AUC] at the maximum recommended human dose of 1.2 grams per day) are inconclusive as to the carcinogenic potential of ribavirin. However, the studies suggested that chronic ribavirin exposure might be related to an increased incidence of vascular lesions (microscopic hemorrhages in mice) and retinal degeneration (in rats). Furthermore, results of a chronic feeding study with ribavirin in rats, at doses of 16-100 mg/kg/day (estimated human equivalent of 2.3-14.3 mg/kg/day, based on body surface area adjustment), suggest that ribavirin may induce benign mammary, pancreatic, pituitary and adrenal tumors.

Ribavirin: Summary of Reproductive Toxicity Study Findings

Ribavirin has demonstrated significant teratogenic and/or embryocidal potential in all animal species in which adequate studies have been conducted. Teratogenic effects have been seen after daily oral doses of 0.3 and 1.0 mg/kg in the rabbit and rat, and after single oral doses of 2.5 mg/kg or greater in the hamster[2]. Malformations of the skull, palate, eye, jaw, limbs, skeleton, and gastrointestinal tract were evident. The incidence and severity of the teratogenic effects generally increased with escalation (increases) of the drug dose. Viability of the fetuses and offspring is typically reduced.

The results of the segment I study in the CD-1 mouse, suggest that ribavirin may produce significant dose and time dependent toxic responses in the testes, including decreases in spermatid concentration, increases in abnormal sperm morphology, and germinal epithelia necrosis. However, fertility studies conducted in male and female SD rats revealed no significant effects of ribavirin on reproductive behaviors or any indices of fertility when the drug was administered for 2-12 weeks prior to mating (females and males; high doses of 10 and 160 mg/kg/day). In a peri- and post-natal exposure study, ribavirin administration at doses up to 1.0 mg/kg/day was without significant adverse effects on pregnant SD rats or their offspring when exposure began after the period of organogenesis and continued through weaning.

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[2] Open literature reports.

Clinical studies with ribavirin administration to pregnant women have not been performed. It should be assumed that ribavirin may cause fetal harm in humans.

Interferon alfa-2b: Summary of General Toxicology Findings As stated previously, interferon alfa-2b is currently approved for IV, SC or IM administration in doses ranging from 2-30 million IU 3x/week, for the treatment of hepatitis B and C, NANB/C hepatitis, hairy cell leukemia, AIDS-related Kaposi's sarcoma and, external condylomata acuminata. The adverse events most commonly associated with interferon use include; fever and flu-like symptoms (headache, nausea, myalgia, fatigue, anorexia and vomiting), granulocytopenia, cardiovascular effects (hypotension, arrhythmia and tachycardia), central nervous system effects (depression, confusion and other changes in mental status), pulmonary infiltrates and, hepatotoxicity (indicated by elevations in serum ALT and/or AST, bilirubin, LDH activity, alkaline phosphatase and prothrombin time, and by decreases in serum albumin and protein). The incidence and severity of the adverse effects appear to increase with the administered dose of interferon alfa-2b and increased duration of therapy.

In preclinical toxicology studies in golden Syrian hamsters and rhesus monkeys, administration of various of the interferons was associated with decreased body weight, decreased food consumption and bone marrow suppression. High-dose chronic exposure (up to 90 fold higher than the maximum recommended clinical dose given daily) in rhesus monkeys was not tolerated for greater than 1 month due to the development of vascular leak syndrome.

Interferon alfa-2b: Summary of Reproductive Toxicology Findings When administered to pregnant female rhesus monkeys, interferon alfa-2b had abortifacient effects at all doses tested (7.5-30 million IU/kg). Furthermore, in non-human primates, interferon administration resulted in alterations in the female menstrual cycle, changes in reproductive behaviors and reductions in serum sex steroid levels. Studies in pregnant rhesus monkeys and golden Syrian hamsters, demonstrated an increase in fetal loss in hamsters treated with Infergen (a geneticly engineered consensus interferon) at doses of greater than 150 µg/kg/day, and in rhesus monkeys at doses of 3 and 10 µg/kg/day. The Infergen toxicity profile described is consistent with the known toxicity profile of other alfa interferons.

There have been no controlled clinical trials in pregnant women or in fertile men to determine the potential effects of interferon administration on human reproduction. It should however, be assumed that interferon may have adverse reproductive effects in the human.

NON-CLINICAL PHARMACOKINETICS AND ADME STUDIES

Ribavirin: The results of multiple pharmacokinetic and ADME studies conducted in the mouse, rat and dog, suggest that ribavirin when given orally either as a solution or in capsule form is well absorbed with an approximate bioavailability (redacted) whereas bioavailability in humans has been estimated (redacted). The data for the 3 animal species, suggest that ribavirin reached maximal levels in the plasma or serum within 1-2 hours of dosing, and decayed with an initial half-life of between 4-10 hours. Drug levels were comparable for male and female animals, and for drug naive or previously drug treated animals (although slight changes in AUC values were evident in rats and dogs following repeat ad-ministration). After acute doses, C_{max} and AUC_{24} values increased in a nearly linear manner with increases in dose, although a slight reduction in systemic exposure was evident in the rat and dog at the highest

doses tested. The data suggest that absorption of ribavirin from the gastrointestinal tract may be reduced at high doses, possibly due to saturation of a carrier transport.

The sponsor has recently submitted the results of two toxicokinetics studies conducted in the mouse and rat. These studies were conducted in the same animal strains and at the same drug doses as were used in the oncogenicity studies of ribavirin. More importantly, however, was the fact that a newly developed more sensitive and more specific drug assay was used than in the previous studies (the new assay being specific to the parent drug structure versus the two primary metabolites [i.e., the deribosylated and triazole carboxamide metabolites). The results of these studies suggest that in the previously reported oncogenicity studies of ribavirin, relative interspecies 24 hour systemic drug exposure levels (at the maximum doses tested in animals versus the recommended 1200 mg clinical dose) were approximately 130% and 20% in the mouse and rat, respectively.

Tissue levels of radioactivity were nearly identical for male and female animals of each species, and were generally much higher than levels noted in the plasma and/or serum. Tissue levels of radioactivity were highest in the gastrointestinal tract, liver and kidneys (apparently related to the organs of absorption, metabolism and excretion of ribavirin). However, an exception to this was evident in the reproductive tissues of both male and female animals, which showed particularly high levels of drug following acute or repeat dose administration (the highest levels of radioactivity detected [per gram of tissue] were in the prostate of the dog). The lowest levels of radioactivity were generally detected in brain tissue.

The primary route of drug elimination was in the urine, with 50-100% of the administered radioactivity being eliminated within 24-48 hours of dosing. Approximately 5-20% of the administered radioactivity was recovered in the feces, and 10% was retained in the carcass at 24 hours after dosing (depending on the species). The entrance of ribavirin into red blood cells was somewhat delayed versus distribution of radioactivity in the plasma, suggesting that the red blood cell membrane may be semi-permeable to the passage of ribavirin and that red cells may serve as a drug reservoir (with delayed release) following drug withdrawal.

Comment: As discussed in the toxicology section of this review, ribavirin has significant adverse effects on rapidly proliferating tissues (lymphoid tissues, mucosa, spleen and testes) and those tissues with high cellular metabolism (heart, liver and secretory cells of the intestinal mucosa). The results of the pharmacokinetic and ADME studies of ribavirin, suggest that the affected tissues are also the primary sites of drug deposition after oral dosing.

Interferon alfa-2b: Pharmacokinetic studies of various of the interferons have been conducted in golden Syrian hamsters, rhesus monkeys and other species. These studies demonstrated rapid absorption following SC injection. Peak serum concentrations were observed at 1 and 4 hours following administration in golden Syrian hamsters and rhesus monkeys respectively. Subcutaneous bioavailability was high in both species, averaging 99% in golden Syrian hamsters and 83-104% in rhesus monkeys. Clearance of drug was approximately 2.0 mL/minute/kg in golden Syrian hamsters and 0.7-0.9 mL/minute/kg in rhesus monkeys. Plasma/serum drug clearance was due predominantly to catabolism and excretion by the kidneys. The terminal half-life of the interferons following SC dosing was approximately 1.5 hours in golden Syrian hamsters and 3.5 hours in rhesus monkeys. Upon 7-day multiple SC dosing, no accumulation of serum levels was observed in golden Syrian hamsters. All interferons have been shown to be highly species specific.

SUMMARY

The sponsor is requesting approval to market ribavirin capsules (REBETOL®; 1-1.2 g/day; PO) in combination with interferon alpha-2b (INTRON A®; 3 MIU TIW; SC Inj.) for the treatment of chronic active Hepatitis type C infection in patients who have relapsed following a previous course of interferon mono-therapy. The proposed duration of combination drug administration in the treatment of Hepatitis type C infection is 6 months.

Interferon alpha (INTRON A®, and related interferons: ROFERON®, WELLFERON®, INFERGEN®) has been previously approved for use at the proposed dose and route of administration for the treatment of Hepatitis type C infection. Since no change in the indication or use of interferon alpha has been proposed in this NDA, the reader is referred to the PLA review (No. 994) for a comprehensive review of the toxicology of INTRON A® brand of interferon alpha-2b. In contrast, ribavirin is currently only approved as an aerosol product (VIRAZOLE®; NDA 18-859) for use in the treatment of Respiratory Syncytial Virus (RSV) infection in the pediatric population. No oral ribavirin containing product is currently available for use in the US. Therefore, this NDA review has focused on the repeat-dose toxicology, reproductive toxicology, genotoxicity and carcinogenicity, pharmacokinetic and ADME studies related to the oral administration of ribavirin (REBETOL®).

The sponsor has submitted results of multiple general toxicology, reproductive toxicology and pharmacokinetic studies in support of this NDA. The results of these studies clearly indicate that when ribavirin is administered at fairly high doses it has significant adverse effects on many rapidly proliferating tissues (lymphoid tissues, mucosa, spleen and testes) and/or those tissues with high cellular metabolism (heart, liver and secretory cells of the intestinal mucosa). Of special concern is an apparent inhibition of the protein synthetic and/or metabolic capacity of the liver, as demonstrated by reductions in multiple serum proteins, albumin and globulins, and elongations of PT and APTT times. Reductions in serum ALT levels evident in several species of non-hepatitis infected animals used in the toxicology studies, and as reported among the Hepatitis type C infected patients treated with ribavirin, may have been due to a toxic inhibition of liver function with resultant decreases in ALT synthesis and release. Results of the pharmacokinetic/ADME studies suggest that the effected tissues are also the primary sites of drug deposition following the oral administration of ribavirin.

Ribavirin demonstrated significant teratogenic and/or embryocidal potential in all animal species in which adequate reproductive toxicology studies were conducted. Teratogenic effects were seen after daily oral doses of 0.3 and 1.0 mg/kg in the rabbit and rat, and after single oral doses of 2.5 mg/kg or greater in the hamster[3]. Malformations of the skull, palate, eye, jaw, limbs, skeleton, and gastrointestinal tract were evident. The incidence and severity of the teratogenic effects generally increased with escalation (increases) of the drug dose. Viability of the fetuses and offspring was typically reduced.

It appears likely that these potentially serious adverse effects of ribavirin have only now become evident, because of the poor study conduct (incomplete gross and/or microscopic pathology, and clinical chemistry/hematology) and the inappropriately low test doses (selected by the drug sponsor) used in previous toxicity studies of ribavirin. The newly submitted toxicology, pharmacokinetic and ADME studies of ribavirin, are in direct response to multiple previous recommendations and requirements made by agency personnel for the provision of additional safety and toxicity information for this drug product.

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[3] Open literature reports.

Based on the available toxicology study results, it is recommended that the Product Label (and Patient Information Sheet) for the combination drug product (i.e., INTRON A®/ REBETOL®), clearly reflect the potentially serious nature of the adverse reproductive and hepatotoxic effects observed in multiple animal species.

CONCLUSIONS

Based on the available animal toxicology and pharmacokinetic data for orally administered ribavirin, it appears relatively safe to approve the combination drug product (interferon alfa-2b [INTRON A] and ribavirin [REBETOL]) for the treatment of chronic active hepatitis due to infection with Hepadnaviridae Type C virus.

RECOMMENDATIONS

1) Based on the availability of a new and more specific assay procedure, which is capable of differentiating between the parent drug molecule and its two primary metabolites, it is recommended that the sponsor perform new pharmacokinetic/toxicokinetic studies of the drug exposure levels which were achieved in the acute-repeat dose and reproductive toxicology studies. These studies will be used to better define the relative interspecies sensitivity to the toxic effects of ribavirin and, will be incorporated into future product label revisions.

2) The in vivo carcinogenicity studies and in vitro/in vivo mutagenicity studies performed with ribavirin were reviewed by the CDER Executive CAC (Center for Drug Evaluation and Research - Carcinogenicity Assessment Committee) in a session on 28 April 1998. It was the conclusion of the committee based on the positive genotoxic effects seen with ribavirin in multiple assay systems, the lack of adequate in vivo rodent carcinogenicity data, and the extended period of human drug exposure (treatment regimen of 6 months duration), that: a) the product label for ribavirin should indicate that it may be a potential carcinogen (see the Carcinogenesis and Mutagenesis section of the proposed product label as contained in Appendix E of this document), and b) the sponsor, as part of a Phase 4 Post-Marketing Agreement, should be required to perform additional in vivo animal studies to assess the carcinogenic potential of ribavirin (See Appendix F of this document for a prioritization of the Sponsor's Phase 4 Commitment).

3) Pre-clinical toxicology data indicate that ribavirin induces a significant degree of anemia (due to direct hemolytic effects and by mild to moderate suppression of the bone marrow), reticulocytosis, and lymphoid tissue atrophy following high dose acute administration or, low dose repeat administration. The anemia is generally reversed within a few weeks following the cessation of ribavirin administration. Similarly, the administration of interferon has been associated with anemia, due to suppression of the bone marrow (an effect which has been seen in multiple animal species and man).

Since both study medications are known to induce hematologic abnormalities with anemia (both by the same and complimentary modes of action), the potential for a synergistic toxicity (i.e., anemia or other blood dyscrasia with earlier onset or greater magnitude of effect) should be clearly identified in the product label. It is recommended that the product label clearly state the need for early and regular monitoring for an increased degree of anemia and/or the onset of delayed anemia.

4) The results of multiple studies suggest that the administration of ribavirin may be associated with reductions in serum protein, albumin and ALT

levels among dogs dosed at 20 mg/kg/day and among rats dosed at 160 mg/kg/day (smaller effects were sometimes evident among animals dosed at lower doses; estimated human equivalent doses of 10 and 23 mg/kg/day [based on body surface area conversion]). Histologic changes evident in either the liver or kidneys were not consistent with these changes. The pattern of the data suggested that the changes in serum protein, albumin and ALT levels may have resulted from a ribavirin induced inhibition of the synthetic capacity of the liver.

Hepatotoxicity and hepatic failure has been noted in patients being treated with interferon. The incidence and severity of the adverse effects appears related to increases in the administered dose and duration of treatment. Hepatotoxicity due to interferon alfa-2b is typically indicated by elevations in serum ALT and/or AST, bilirubin, LDH activity, alkaline phosphatase and prothrombin time, and by decreases in serum albumin and protein concentrations.

It is recommended that the product label clearly state the need for regular monitoring of hepatic functioning, but that the measurement of ALT and AST (as the most common LFT'S) may not accurately reflect liver functioning or injury since the concurrent use of ribavirin may artificially suppress these values.

5) Ribavirin has demonstrated significant teratogenic and/or embryocidal potential in all animal species in which adequate studies have been conducted. Teratogenic effects have been seen after daily oral doses of 0.3 and 1.0 mg/kg in the rabbit and rat, and after single oral doses of 2.5 mg/kg or greater in the hamster[4]. Malformations of the skull, palate, eye, jaw, limbs, skeleton, and gastrointestinal tract were evident. The incidence and severity of the teratogenic effects generally increased with escalation (increases) of the drug dose. Viability of the fetuses and offspring is typically reduced. In addition, interferon demonstrated significant dose-related abortifacient effects when administered to pregnant rhesus macaques.

Clinical studies with ribavirin or interferon administration to pregnant women have not been performed. It should be assumed that ribavirin or interferon may cause fetal harm in humans.

As noted in the Pharmacokinetics section of this review, ribavirin is concentrated in erythrocytes and other tissues, and undergoes extended elimination. Therefore, the minimum interval following exposure to ribavirin before pregnancy may be safely initiated in the human is unknown, although an interval of 3-5x the terminal elimination half-time should be considered (i.e., 3-5x the erythrocyte $t_{1/2}$ of 40 days = 120-200 days or approximately 6 months).

It is recommended that the product label clearly state the need for determining pregnancy status prior to the initiation of drug treatment, in addition to regular monitoring for pregnancy during treatment and for 6 months following treatment. The product label should clearly state the need for the use of effective contraception methods by both female and male patients being treated interferon and ribavirin.

6) The results of 2 multi-year studies of ribavirin conducted in mice and rats suggest that chronic drug exposure might be related to an increased incidence of vascular lesions (microscopic hemorrhages in mice) and retinal degeneration (in rats). It is recommended that this adverse animal finding be included in the combination product label, along with a recommendation for the regular monitoring of patients for neurologic status and retinal changes.

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[4] Open literature reports.

/S/

David E. Morse, Ph.D.
Reviewing Pharmacologist

Concurrences:

HFD-530/Dir./HJolson
HFD-530/ADDir./WDempsey 5/15/98
HFD-530/TLPharm/JGFarrelly 5/12/98
HFD-530/Pharm/DMorse

HFD-530/Disk Copy

cc:
HFD-530/NDA 20-903
HFD-530/Division File
HFD-340/
HFD-530/CSO/TCrescenzi
HFD-530/Pharm/DMorse
HFD-530/MO/RFleischer, TNguyen
HFD-530/Chem/
HFD-530/Micro/

APPEARS THIS WAY
ON ORIGINAL

Appendix A: Acute - Chronic Toxicity Studies

Summary of General Toxicology Findings:

Pre-clinical animal toxicology data and previous human experience indicate that ribavirin induces a significant degree of anemia (due to a direct hemolytic effect and suppression of the bone marrow), reticulocytosis, and lymphoid atrophy following high dose, acute administration or low dose, repeat administration. The anemia and lymphoid effects are generally reversed within weeks following the cessation of ribavirin administration. Study data suggest that accumulation of ribavirin occurs during repeat dosing procedures, but that it generally stabilizes 1-3 weeks following the start of dosing.

As noted in the sections which follow, the administration of ribavirin was associated with slight reductions in serum protein, albumin and ALT levels, among dogs dosed at 20 mg/kg/day and among rats dosed at 160 mg/kg/day (smaller effects were sometimes evident among animals dosed at lower levels; estimated human equivalent doses of 10 and 23 mg/kg/day [based on body surface area conversion]). The results of these studies, in conjunction with other submitted materials, suggest that when ribavirin is administered at fairly high doses it has significant adverse effects on many rapidly proliferating tissues (lymphoid tissues, mucosa, spleen and testes) and/or those tissues with high cellular metabolism (heart and liver). Of concern is a potential inhibition of the protein synthetic and/or metabolic capacity of the liver, as demonstrated by changes in multiple serum proteins, albumin and globulins, and elongations of PT and APTT times. Histologic abnormalities evident in either the liver or kidneys were not consistent with these changes. The data suggest that the changes in serum protein, albumin and ALT might be the result of decreased synthesis. Thus, while serum protein, albumin and transaminase levels generally remained near-normal throughout treatment, the observed decreases in serum protein, albumin and ALT levels may have resulted from a ribavirin induced inhibition of liver synthetic capacity.

Reductions in serum ALT levels evident in the rat and other species of non-hepatitis infected animals used in the toxicology studies, and as reported among Hepatitis "C" infected patients treated with ribavirin, may be due to a toxic inhibition of liver function with resultant decreases in ALT synthesis and release. Results of ADME studies in multiple species suggest that the affected tissues are also the primary sites of drug deposition following oral dosing with ribavirin. Based on these new results, it is recommended that the product label for the approved formulation of ribavirin reflect the potentially serious nature of the toxic effects observed in the liver of the rat and dog.

It appears likely that these potentially serious adverse effects of ribavirin have only now become evident, because of the poor study conduct (incomplete gross and/or microscopic pathology, and clinical chemistry/hematology) and the inappropriately low test doses used in previous toxicity studies of ribavirin [redacted]. The newly submitted toxicology, pharmacokinetic and ADME studies of ribavirin, are in direct response to multiple previous recommendations and requirements made by agency personnel for the provision of additional safety and toxicity information for this drug product.

Toxicity Studies Summary:

MOUSE

1) **A 90-Day Oral Gavage Range-Finding Study In Mice With Ribavirin,** GLP,

RAT

2) **Ribavirin - 30 Day Dietary Toxicity Study In Rats,** Study No. 451199, GLP,
Initiation: 21 Jan. 1993, Ribavirin Lot# 05500787 (R-17),
3) **A 90-Day Oral Gavage Range-Finding Study In Rats With Ribavirin,** GLP,

4) **Ribavirin: 52 Week Dietary Toxicity Study In Rats With 26 Week Interim Kill,** Study No. 451204, GLP,
5 May 1993, Ribavirin Lot# BR-17,

DOG

5) **Ribavirin - 30 Day Pilot Oral (Gavage) Toxicity Study In The Beagle Dog,** Study No. 895/001, GLP, Study
Initiation: 15 Jan. 1993, Ribavirin Lot# 05500787 (R-17),
6) **Ribavirin: 52 Week Oral (Gavage) Toxicity Study In The Beagle Dog With An Interim Necropsy After 26 Weeks,** Study No. 895/002, GLP,
23 March 1993,
Ribavirin Lot# BR-17,

Toxicity Study Reviews:

MOUSE

1) **A 90-day oral gavage range-finding study in mice with ribavirin** (GLP study conducted

This was a dose-finding study for a subsequent mouse carcino-genicity study. Six groups of CD-1 mice (10/s/g) were dosed daily with vehicle, 35, 75, 150, 300 or 600 mg/kg ribavirin by gastric intubation for 3 months. Physical observations, body weights and food consumption were measured in all animals pretest and throughout the study. Hematology was performed on all animals surviving to the end of the study. Blood samples were also taken for pharmacokinetic data; these data were not included in this study. All animals were necropsied; histopathology was confined to examination of testes and epididymides of all males except the 35 mg/kg group.

Premature mortality approached 50% at 300 mg/kg (5/10 males, 4/10 females) and 100% at 600 mg/kg (all but one female). Most of the deaths occurred late in the study (weeks 12-13). Specific pathologic changes leading to death were not identified, but the mortality was considered by the sponsor to be related to ribavirin administration. Based on hematologic changes observed in survivors from the 300 mg/kg group (see below), a tentative diagnosis of severe anemia is plausible. An additional 35 mg/kg female was found dead during week 7. Cause of death was not immediately apparent, but the death was not considered by the sponsor to be drug-related.

General behavioral observations included scabbing, particularly of the ear region, and staining of fur at doses of 150-600 mg/kg. In the 600 mg/kg group, body weight gains were decreased by 9-12% beginning at week 4 and continuing to week 12, when the decrement reached up to 34%. Animals in the

300 mg/kg group had sporadic body weight deficits of similar magnitude during weeks 8-12. There no body weight changes at or below 150 mg/kg. Food consumption (g/kg body weight) was generally maintained at or above control levels in all groups.

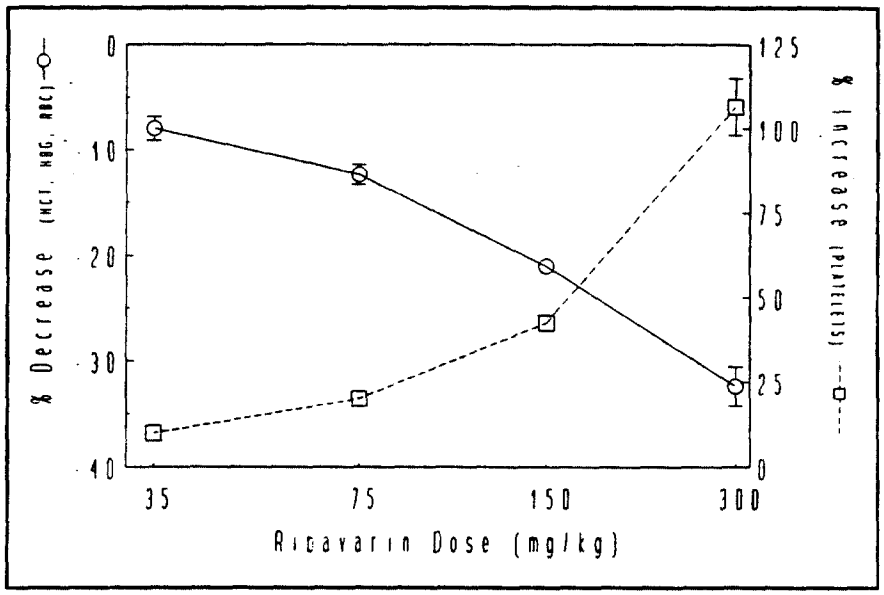

Figure 2 - Mouse Hematology Data

Anemia and thrombocytosis were observed at all ribavirin doses. There were dose-related decreases in hemoglobin, hematocrit and red cell counts and dose-related platelet increases which reached statistical significance in all but the 35 mg/kg group. Only one 600 mg/kg female was available for hematology; this animal was not included in the statistical analysis for obvious reasons. Mice treated with 300 mg/kg also had a 40% increase in reticulo-cyte counts and 10% increases in mean cell volume and hemoglobin. Differential counts appeared to be unaffected at 300 mg/kg, but these animals did have increases in nucleated red blood cells and poikilocytes (differential counts and morphology were performed only in the control and 300 mg/kg groups).

Spleen weights were increased 2 to 4-fold in the 300 mg/kg group and by 50-75% at 150 mg/kg ribavirin. Gross splenic enlargement was noted in 6 animals in the 300 mg/kg group. Testicular weights were decreased by 30 and 16% in males treated with 300 and 150 mg/kg, respectively. Histopathology revealed evidence of bilateral degeneration of the testicular germinal epithelium with epididymal oligospermia in the majority of males dosed with 150 mg/kg or more (vehicle 2/10, 75 mg/kg - 0/10, 150 mg/kg - 7/10, 300 mg/kg - 8/10, 600 mg/kg - 9/10 animals).

The lowest dose group (35 mg/kg) appeared to be near the no drug effect threshold. There was a trend in this group toward the same hematologic changes observed at the higher doses - loss of erythrocytes and increases in platelets. Testicular changes were observed beginning at 150 mg/kg. This group was probably close to the maximum tolerated dose, as higher doses produced severe anemia and unacceptable mortality.

RAT

2) **Ribavirin - 30 Day Dietary Toxicity Study In Rats**, Study No. 451199.
Status: GLP
Study Site:
Study Initiation: 21 Jan. 1993
Compound Tested: Ribavirin Lot# 05500787 (R-17),
Doses Tested: 0, 10, 40, 160 and 320 mg/kg/day
Dose Volume and Route: dietary admixture, oral
Solvent: ground rodent diet
Species, Strain, Sex: male & female SD rats, age 6 weeks; weight range: male = 120-145 grams; female = 83-103 grams, 10 animals/sex/dose.
Test conditions: Animals were randomly assigned to treatments. Ribavirin was available continuously as a dietary admixture for a period of 30 days. Physical signs, mortality, body weight and food consumption were monitored regularly throughout the study. Hematologic analyses and clinical chemistries were performed after 31 days of drug administration. Ophthalmologic examinations were performed at baseline, and on treatment days 15 and 26. Gross and microscopic examinations of all standard tissues were performed at the termination of dosing.

Mortality: All animals from the high dose group (320 mg/kg/day) were sacrificed in extremis on day 10 of drug administration, with clinical signs of extreme weight loss, inappetence, pilo-erection and subdued behavior. An additional 3 males and 4 females from the high-intermediate dose (160 mg/kg/day) group died or were euthanized prior to the conclusion of the study. At necropsy, the most common abnormalities noted in these animals included a flaccid appearing heart, reddening and/or mottling of the lungs, fluid accumulation in the thoracic cavity, and enlargement of multiple lymph nodes.

There were no premature deaths among animals in the control, low or low-intermediate dose groups.

Clinical Signs: Other than signs noted among the moribund animals, surviving animals from the high-intermediate dose group displayed multiple signs including, ataxia, abnormal respiration, hunched posture, hypothermia and piloerection. There were no obviously drug related clinical signs noted among animals in the 2 lowest dose groups.

Body Weight and Food Consumption: Ribavirin caused a dose related reduction (group mean reductions in weight gain of 20-140% of the concurrent control) in body weight gain, or a loss of body weight, during the first week of dosing. Changes in weight gain were evident in both male and female test animals. Weight gain among all animals from the intermediate dose groups (i.e, 40 and 160 mg/kg/day) remained suppressed throughout the drug dosing interval, such that at termination, absolute body weight of the affected animals was moderately to markedly reduced (reduction in weight gain of 20-70%). Following the initial week of drug administration, weight gain among animals from the low dose group was comparable to the controls.

Among the drug treated animals, food consumption was reduced in a dose related manner which closely paralleled the changes noted in body weight gain. Food consumption was reduced throughout the dosing interval by an average of 30-50% among the animals treated at 160 mg/kg/day (versus the concurrent controls). Among the study animals dosed at 10 or 40 mg ribavirin/kg/day, average reductions in food intake were approximately 10-20% of control.

Dietary Drug Intake: The dietary intake of ribavirin for all dose groups was generally within 20% of the assigned dose (computation of drug intake and adjustment of drug-diet concentration were performed weekly). Drug

concentration was measured in specimens taken at the time of necropsy (time after lights-out was not specified), results indicating that plasma levels of ribavirin generally increased in a less than linear manner with the nominal dose.

Dose (mg/kg)		Plasma Drug Levels (µMolar) at Necropsy 0	10	40	160	320
Sex						
♂		BLD	1.8	11.4	79.4	108.7
	N=	(3)	(10)	(10)	(8)	(9)
♀		BLD	2.4	26.3	73.2	118.1
	N=	(3)	(10)	(10)	(6)	(10)

BLD = Below Limit of Detection (LD = 0.05 µMoles/liter)

Ophthalmoscopy: There were no apparent drug related effects.

Hematology and Clinical Chemistry: Among drug treated males, significant reductions (40% versus control) in mean hemoglobin concentration and hematocrit were evident at the conclusion of dosing. Reductions appeared dose related and were accompanied by a decrease in RBC counts (40%) in animals dose at 160 mg/kg/day, while animals dosed at 10 and 40 mg/kg/day displayed slight increases in MCH and MCV (5-10%). Similar reductions (40-50%) in hemoglobin, hematocrit and RBC counts were seen in surviving females from the 40 and 160 mg/kg/day treatment groups. However, in contrast to the effects seen in males, MCH and MCV were significantly reduced (5-10%) among female animals from the 10 and 40 mg/kg/day treatment groups.

Total white cell, lymphocyte and eosinophil counts were reduced (near 50%) among all surviving high-intermediate dose treated animals at the time of necropsy. The bone marrow appeared normal among all drug treated animals except 4 (2 male and 2 female) from the 160 mg/kg/day group, which showed moderately reduced cellularity of the marrow.

Changes in serum chemistry noted at the conclusion of dosing, included: a) decreases (10-25%) in serum protein, albumin, creatinine and calcium in females dosed at 160 mg/kg/day (high-intermediate dose), b) a reduction in serum ALT levels among males dosed at 160 mg/kg/day (approx. 50% versus the controls), and c) increases in serum AST levels among females dosed at 160 mg/kg/day (approx. 45% versus the controls). Prothrombin time was slightly increased (approx. 25% [mean of 4 sec.]) among the high-intermediate dose treated female animals. There were no other changes in any hematologic or serum chemistry parameter noted.

Gross and Microscopic Pathology: At necropsy, increases in the absolute and/or relative weight of the heart and lungs was seen in both male and female animals treated with ribavirin at 160 mg/kg/day. This effect was particularly pronounced in 5 males and 4 females treated at 160 mg/kg/day, but was also noted in 1 male and 3 female animals which had received ribavirin for 10 days at 320 mg/kg/day. Besides the increase in organ weight, the heart muscle of the affected animals appeared flaccid and dilated on gross examination. Microscopic examination of the tissues showed significant evidence of cardiomyopathy in 7 males and 6 females from the 160 mg/kg/day treatment group, and in all but 1 male (i. e., 9 males and 10 females) from the 320 mg ribavirin/kg/day group. More frequent and severe alveolitis was noted in these same animals than was evident in animals from any of the other treatment groups. Fluid was evident in the thoracic cavity of 3 male and 6 female animals treated at 160 mg ribavirin/kg/day.

Decreases in both absolute and relative weight of the thymus were noted in

male and female animals from the 160 mg/kg/day group, while spleen weight was increased. Spleen weight was also increased in male animals dosed at 10 and 40 mg/kg/day, the changes generally appearing dose related. Microscopic examination of multiple lymphoid tissues revealed generalized atrophy (thymus and lymph nodes) with loss of germinal centers, macrophage and other inflammatory cell infiltrates, and congestion. Extramedul-lary hemopoiesis was decreased or absent in the spleen of multiple animals dosed at 40 mg/kg/day and above.

Multifocal necrosis was seen in all segments of the intestines of animals from the two high dose treatment groups (i.e., 160 and 320 mg/kg/day). Necrosis was occasionally accompanied by mucosal atrophy and goblet cell hyperplasia in some animals (the effect being dose related). Mucosal cysts were evident in sections of the colon of 1 and 5 animals from the 160 and 320 mg/kg/day dose groups, respectively.

Microscopic examination of multiple other tissues showed evidence of drug related injuries, including: a) centrilobular necrosis of the liver (7 females dosed at 160 mg/kg, and 3 females and 1 male dosed at 320 mg/kg [for 10 days]), b) periportal fibrosis of the liver (4 males dosed at 160 mg/kg), c) atrophy of the salivary glands (4 males and 5 females dosed at 160 mg/kg, and 7 males and 9 females dosed at 320 mg/kg), d) decreased secretion of the seminal vesicals (2 and 4 males dosed at 160 and 320 mg/kg), and e) testicular tubular epithelial atrophy (1 and 5 males treated at 160 and 320 mg/kg).

All remaining gross and/or microscopic lesions appeared to be randomly distributed among the treatment and control groups.

Comments: 1) Anemia and lymphopenia have been observed in nearly all animal species (including man) following acute or repeat dosing with ribavirin. These effects typically become apparent within 1-2 weeks of ribavirin adminis-tration. The degree of anemia and lymphopenia appears to be both dose and time dependent.

2) The results of the present study suggest that ribavirin when given at relatively high doses (> 40 mg/kg/day) has significant adverse effects on rapidly proliferating tissues (lymphoid tissues, the gastro-intestinal mucosa, spleen, bone marrow, testes, seminal vesicals, and the salivary glands) and/or those tissues with the highest rate of cellular metabolism (heart, liver and secretory cells of the gastro-intestinal mucosa).

Significant dose related cardiomyopathy and periportal hepatic fibrosis (males) and/or necrosis (females) was evident in animals dosed at 160 mg/kg/day (estimated human equivalent dose of 22.8 mg/kg/day, based on body surface area dose adjustment), or above. These organs are likely to receive the highest levels of drug exposure following oral administration, due to the receipt of periportal and central venous blood flow.

Cardiac and hepatic injuries have not been noted in the previously submitted GLP toxicology studies. Therefore, it is recommended that all oral repeat dose clinical trials with ribavirin include follow-up evaluations of cardiac and hepatic function.

3) As noted above and in comments to the preceding study, the administration of ribavirin was associated with reductions in serum proteins and albumin and an increased prothrombin time among the female animals dosed at 160 mg/kg/day. ALT levels were reduced and AST levels increased (approx. 50% each) among the male and female animals, respectively. As in the dog study, no

abnormalities were noted that might suggest a mechanism of increased protein/albumin loss, therefore suggesting that the changes in serum protein/albumin might be the result of decreased synthesis in the liver. Additional markers of hepatic function including serum CPK levels and mitochondrial citrate synthase or cytochrome oxidase were not measured.

It should be remembered that the proposed mechanisms of action for ribavirin are through incorporation into, or inhibition of the synthesis of RNA, with a resultant failure in the RNA message. Incorporation of ribavirin in the cellular genome has not been reported. Thus, if the DNA remains intact, and messenger RNA turnover is high, the removal of ribavirin from the system should result in recovery of impaired functions (assuming that cellular function has not been so compromised as to preclude the minimal level of synthetic activity which is necessary for cellular survival).

4) The results of this study, suggest that the maximal survivable dose for a 12 month toxicity study in the SD rat likely falls between 40 and 160 mg/kg/day (when administered as a dietary admixture).

3) **A 90-day oral gavage range-finding study in rats with ribavirin** (GLP study conducted

This was a dose-finding study for a subsequent rat carcino-genicity study. Six groups of Sprague-Dawley CD rats (10 animals /sex/group) were dosed daily with vehicle, 20, 40, 80, 150 or 200 mg/kg ribavirin by gastric intubation for 3 months. Physical observations, ophthalmoscopic examinations, body weights and food consumption were measured in all animals pretest and throughout the study. Hematology and clinical chemistry were performed on 5 animals/s/g at the beginning and end of the study. All animals were necropsied; histopathology was confined to examination of testes and epididymides in control and 200 mg/kg males.

There were 5 premature deaths: 1-150 mg/kg female was sacrificed moribund on day 16, 1-200 mg/kg female was found dead on day 78, 1-200 mg/kg male was sacrificed moribund on day 86 and 2 others were found dead, 1 each on days 32 and 83. Autopsy results were unrevealing as to cause of death and there were no other data for these animals (clinical chemistry, etc.) to aid in diagnoses.

Surviving 150 and 200 mg/kg animals displayed alopecia, scabs and sores, mostly of the mouth and dorsal cervical region. The frequency of these observations was increased with duration of treatment, so that these symptoms were present in the majority of animals in these groups by week 13. Body weights in the 200 mg/kg group were decreased by 12-22% relative to controls, beginning at study week 2 and continuing throughout the study. Body weights in the 150 mg/kg group were also sporadically decreased. Food consumption was affected in a parallel manner. There were no effects on body weight gain or food consumption at doses at or below 80 mg/kg. Ophthalmological examinations were unremarkable.

Hematologic measures indicated significant anemia and thrombocytosis at all ribavirin doses except 20 mg/kg, with small, statistically nonsignificant changes in the latter group. Decreases in hemoglobin, hematocrit and erythrocytes were dose-related, as were increased platelet counts. Total white cell counts were decreased by up to 50%, but only at 150-200 mg/kg. Differential counts appeared to be unaffected at 200 mg/kg, but these animals did have slight increases in the number of atypical red cells (nucleated,

poikilocytes, hypochromia - differential counts and morphology were performed only in control and 200 mg/kg groups). Serum potassium was increased by 8-16% at 150-200 mg/kg. SGPT and SGOT were elevated by ~50% at 200 mg/kg, but the changes were not statistically significant.

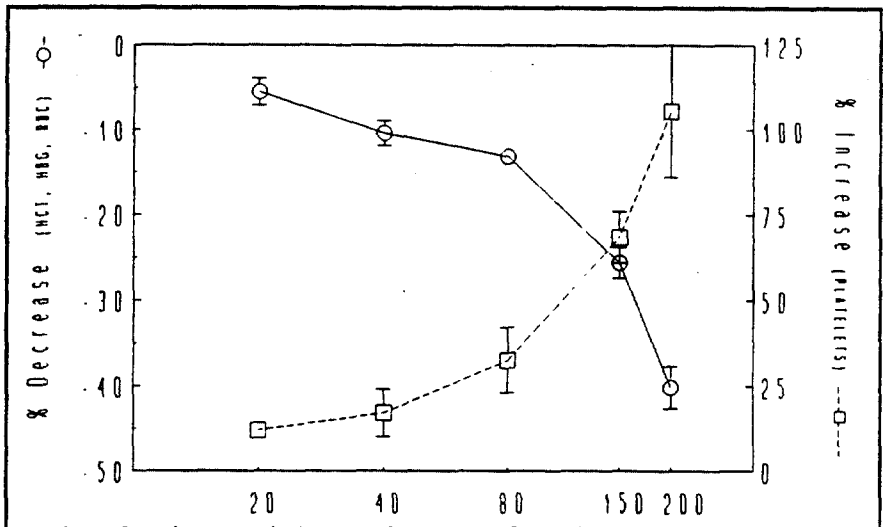

Figure 1 - Rat Hematology Data

The absolute and relative weights of the following organs were increased in 200 mg/kg animals of both sexes: adrenal (13-37%), heart (8-58%) and spleen (13-55%). Necropsy findings included the following: discolored lungs 7/10 males at 200 mg/kg vs. 3 control males, dilated renal pelvis 3/10 males at 200 mg/kg vs. 0 controls, liver discoloration 6/20 at 200 mg/kg vs. 0 controls, cardiac enlargement 2/10 males at 200 mg/kg vs. 0 controls. Microscopic examination of testes and epididymides from controls and 200 mg/kg males revealed no indications of ribavirin-related toxicity.

The data indicate 20 mg/kg as a no effect dose with the 40 mg/kg dose approaching the threshold for significant hematologic effects. The maximum tolerated dose appeared to be near 200 mg/kg, with weight loss, anemia and thrombocytosis evident at this dose level.

4) **Ribavirin: 52 Week Dietary Toxicity Study In Rats With 26 Week Interim Kill**, Study No. 451204.

Status: GLP
Study Site:
Study Initiation: 5 May 1993
Compound Tested: Ribavirin Lot# BR-17,
Doses Tested: 0, 1, 10, 30 and 90 mg/kg/day
Dose Volume and Route: dietary admixture, oral
Solvent: ground rodent diet

Species, Strain, Sex: male & female SD rats, age 6-7 weeks; weight range: male = 170-215 grams; female = 119-159 grams, 50 animals/sex/dose (an additional 10 animals/sex/dose were included in a satellite study for the periodic assessment of plasma drug levels).

Test conditions: Animals were randomly assigned to treatments. Ribavirin was available continuously as a dietary admixture for a period of 26 or 52 weeks. Physical signs, mortality, body weight and food consumption were monitored regularly throughout the study. Hematologic analyses and clinical chemistries were performed after 4, 13, 26, 39 and 50-52 weeks of drug exposure in a subset of animals. Ophthalmologic examinations were performed at baseline, and

at approximately 4-5 week intervals throughout the remainder of the study. Gross and/or microscopic examinations of all standard tissues were performed at the termination of dosing on all animals from the control and high dose groups and all premature deaths.

Results after 26 weeks of drug administration

Mortality: There were a total of 15 premature deaths (high dose: 8M/4F; high-intermediate dose: 1F; low-intermediate dose: 2M) during the 26 weeks of drug administration. Clinical signs noted among the affected animals included an increased incidence of peri-oral ulcers and scabbing, but did not include signs suggestive of a mechanism or cause of death.

There were no premature deaths among animals in the control or low dose groups.

Body Weight and Food Consumption: Ribavirin administration caused a dose related reduction in body weight gain (mean reductions in weight gain of 10-40% of the concurrent control) among males and females from the 2 highest dose groups. Among males and females from the high dose group, significant reductions in weight gain were evident after as little as one week of drug administration. Following the initial few weeks of drug administration, weight gain among animals from the high-intermediate dose group was generally comparable to the controls. Weight gain among animals from the low-intermediate and low dose groups was comparable to the controls.

Food consumption was reduced in a dose related manner among male and female animals from the 2 highest dose groups, which closely paralleled the changes noted in body weight gain. Food intake was reduced throughout the dosing interval by an approximate 10% among males and females from the high dose group. Among animals dosed at 30 mg ribavirin/kg/day, decreased food intake was evident during the early weeks of drug administration and sporadically thereafter.

Dietary Drug Intake: Dietary intake of ribavirin was within 20% of the nominal dose level set for each treatment group through-out the study. Drug intake was computed and adjustments of drug-diet concentration performed on a weekly basis.

Plasma drug levels were measured in specimens taken at the time of necropsy (time after lights-out was not specified). Results of these assays indicate that plasma levels of ribavirin generally increased in a slightly more than linear manner with the nominal dose.

		Plasma Drug Levels (µMolar)				
Dose (mg/kg)		0	1	10	30	90
Sex						
♂	WK4	BLD	.028	.367	1.47	7.50
	WK13	BLD	.071	.325	1.46	7.72
	WK15	BLD	.084	.599	1.70	8.96
	WK26	BLD	.092	.740	2.23	10.99
	N=	10	10	10	10	10 or 9
♀	WK4	BLD	.026	.442	1.52	9.03
	WK13	BLD	.073	.454	1.64	9.07
	WK15	BLD	.059	.372	1.79	8.79
	WK26	BLD	.089	1.01	3.23	15.48
	N=	10	10	10	10	10

BLD = Below Limit of Detection (LD = 0.025 µMoles in Plasma)

Ophthalmoscopy: There were no apparent drug related effects.

Hematology and Clinical Chemistry: The administration of ribavirin at a daily dose of 90 mg/kg, resulted in significant reductions (10-40% of the concurrent controls) in hemoglobin concentration, red blood cell counts, hematocrit and mean corpuscular hemoglobin concentration, which were evident after 4 weeks of drug administration and were maintained throughout the 26 week dosing interval. Similar, although somewhat smaller, reductions in red cell parameters were evident among males and female animals dosed at 10 or 30 mg/kg/day. The reductions in red cell parameters appeared dose related among animals dosed at 10-90 mg/kg/day, with animals dosed at 10-30 mg/kg/day showing slight increases in MCV (5-10%).

Total white cell, lymphocyte, basophil and/or eosinophil counts were reduced (up to 50%) among surviving animals dosed at 90 mg/kg/day throughout the drug administration interval. Sporadic reductions in most white cell parameters were also noted among animals treated with ribavirin at doses of 10-30 mg/kg. Despite the significant effects on circulating red and white blood cell parameters, the bone marrow appeared normal among all drug treated animals at the time of necropsy.

Changes in serum chemistry noted at multiple time-points during the drug administration interval, included: a) a reduction (20% versus the controls) in serum ALT levels among males and females dosed at 90 mg/kg/day (weeks 4, 13 and 26 of dosing), b) sporadic reductions in serum ALT levels among animals dosed at 30 mg/kg (males at week 13; females at weeks 13 and 26), c) increases in serum AST levels among male and female animals dosed at 90 mg/kg /day for 4 weeks (no significant effect thereafter), and d) decreases (up to 25%) in serum protein and fluctuations in serum albumin levels (increases and decreases) among males and females dosed at 30 mg/kg/day or greater.[5] Among males, serum chloride levels were reduced slightly after 4 weeks of drug administration at levels of 10 and 30 mg/kg/day (but not at 90 mg/kg/day), while females dosed at 10-90 mg/kg/day for 26 weeks showed increases in serum phosphate levels (significant only among the high dose animals). Prothrombin time was not significantly altered by the administration of ribavirin at any dose tested. There were no other changes in any hematologic or serum chemistry parameter noted.

Gross and Microscopic Pathology: At necropsy, increases in the absolute or relative weight (covariate analyses) of the heart and lungs was seen in both male and female animals treated with ribavirin at 90 mg/kg/day. Thymus weights were significantly reduced among the high dose treated animals. Among females, the mean uterine weight was significantly decreased among animals dosed at 1, 30 or 90 (but not at 10) mg/kg/day. Sporadic, non-dose related, increases in the weight of the spleen, kidneys and liver were evident among ribavirin treated animals from all dose groups. Besides the increase in organ weight, the heart muscle of the affected animals appeared flaccid and dilated on gross examination.

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[5] The administration of ribavirin was associated with reductions in serum proteins and ALT levels, and sporadic increases in serum AST levels, in animals given ribavirin at > 30 mg/kg/day. Since no biochemical (i.e., creatinine, BUN or CPK) or histologic changes (i.e., renal or hepatic) were noted that might suggest a mechanism of increased protein loss, the data suggest that the changes in serum chemistry might be the result of decreased synthesis by the liver. However, this conclusion can not be confirmed based on the available study results.

As noted above, decreases in both absolute and relative weights of the thymus were noted in male and female animals dosed at 90 mg/kg/day, while spleen weight was increased. Microscopic examination of multiple lymphoid tissues revealed generalized atrophy (thymus and lymph nodes) with loss of germinal centers, macrophage and other inflammatory cell infiltrates, and congestion. Extramedullary hemopoiesis was decreased or absent in the spleen of multiple animals dosed at 30 mg/kg/day and above.

Microscopic examination of tissues from the lungs of animals dosed at 30 or 90 mg ribavirin/kg/day, revealed more frequent and severe alveolitis (with macrophage infiltration and pneumonitis) among these animals than was evident in animals from any of the other treatment groups. Hyperplasia of the pulmonary arterioles was also evident among 4/13 males from the high dose group.

All remaining gross and/or microscopic lesions appeared to be randomly distributed among the treatment and control groups.

Results after 50[6]-52 weeks of drug administration

Mortality: There were a total of 45 premature deaths (high dose: 27M/8F; high-intermediate dose: 2F; low-intermediate dose: 3M; low dose: 1M/1F; control: 3F) during the 52 weeks of drug exposure (which includes animals dyeing prior to the mid-study interim sacrifice). Similar to the findings after 26 weeks of drug administration, the only clinical signs noted among the effected animals included an increased incidence of peri-oral and peri-anal ulcers/scabbing and inflammation.

Body Weight and Food Consumption: Ribavirin administration was associated with a significant decrease in terminal body weight and weight gain (mean reductions in weight gain of 30-40%) of male and female animals from the high dose group. Among animals from the high dose group, significant reductions in weight gain were evident after as little as one week of drug administration and remained evident throughout the drug administration interval. Animals from the high-intermediate dose group showed a transient decline in weight gain immediately following dose initiation, although weight gain following several weeks of continuous drug exposure was generally comparable to control. Weight gain among animals from the low-intermediate and low dose groups was comparable to the controls throughout the study.

Food consumption was reduced in a dose related manner among male and female animals from the 2 highest dose groups, particularly early in the drug administration interval. However, by the conclusion of the 50-52 week drug administration period, only the animals from the high dose group continued to display reduced intake of food (5-10% reduction) as compared with control. The changes in food consumption seen among the high-intermediate and high dose animals closely paralleled the changes noted in body weight. Similar to the perturbations in body weight gain seen among the high-intermediate dose animals, decreased food intake among these animals was evident during the early weeks of drug administration and sporadically thereafter.

Dietary Drug Intake: The cumulative and weekly dietary intake of ribavirin was within 2 and 20% of the nominal dose level set for each treatment group in the study, respectively. Plasma and erythrocyte drug levels were measured in in-life and necropsy specimens and are reported in the following tables.

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[6] Due to excessive mortality the high dose treated male animals were terminated at week 50. All remaining study animals were terminated as scheduled during week 52.

Plasma Drug Levels (µMolar) at Necropsy
(data for weeks 4-26 are presented in a previous table)

Dose (mg/kg)		0	1	10	30	90
Sex						
♂	WK26	BLD	.092	.740	2.23	10.99
	N=	10	10	10	10	9
	WK39	---	---	---	2.64	---
	N=	---	---	---	10	---
	WK50	BLD	.10	.97	4.38	13.32
	N=	10	10	9	10	7
♀	WK26	BLD	.089	1.01	3.23	15.48
	N=	10	10	10	10	10
	WK39	---	---	---	2.72	---
	N=	---	---	---	10	---
	WK52	BLD	.05	.86	3.56	16.65
	N=	8	8	10	9	8

BLD = Below Limit of Detection (LD = 0.025 µMoles in Plasma),
--- = No data reported

Mean Erythrocyte Drug Levels (µMolar)

Dose (mg/kg)		0	1	10	30	90
Sex						
♂	WK4	BLD	0.42	3.06	8.20	59.75
	WK15	BLD	BLD	BLD	1.86	12.97
	WK26	BLD	BLD	0.21	2.21	9.39
	WK39	BLD	BLD	2.85	7.45	12.66
	WK50	BLD	BLD	0.64	3.16	5.91
	N=	7-10	9-10	9-10	9-10	6-10
♀	WK4	BLD	BLD	1.65	8.83	59.09
	WK15	BLD	BLD	0.28	3.78	11.00
	WK26	BLD	BLD	0.71	6.43	16.24
	WK39	BLD	BLD	1.43	8.22	23.01
	WK52	BLD	BLD	0.69	7.33	17.95
	N=	8-10	7-10	8-10	9-10	6-10

BLD = Below Limit of Detection (LD = 2.0 µMoles in RBC)

Comments: 1) The data presented below suggest that in the rat, ribavirin is well absorbed after oral administration in the diet and plasma drug levels generally increase in an approximately linear manner with the nominal dose. The data also suggest that a slight increase in plasma drug levels may occur over an extended period of drug exposure. However, timing of sample acquisition is not included in the study report and would be expected to have a significant impact on measured drug levels (i.e., since the majority of diet consumption [and therefore drug intake] occurs shortly after the start of the dark period, samples drawn shortly after this time would be expected to be higher than samples taken at other times [based on a $t_{1/2}$= 4 hrs]; it is therefore not clear whether the variation in the reported values is due to changes in physiologic disposition of the drug or to experimental artifact).

2) Drug levels in the erythrocyte appear to be more variable than levels in the plasma, and do not appear closely associated with the administered dose. The study data suggest that a gradual decrease in the intra-erythrocyte levels of ribavirin may occur during long periods of repeat exposure. While no data exist, the

most likely mechanism for this change may be through a reduction in erythrocyte permeability to ribavirin, since there is no known mechanism for the elimination of ribavirin-triphosphate from the erythrocyte.

Ophthalmoscopy: There were no clear differences in the incidence of ophthalmic lesions in the ribavirin treated and control animals through week 25 of drug administration. However, beginning with week 38 of the study, there was a significant increase in the frequency of increased retinal reflectivity and/or reductions in retinal perfusion among the high dose ribavirin treated animals versus the concurrent controls (increased retinal reflectivity: 9M/19F versus 2M/3F; decreased retinal perfusion: 3M/7F versus 0M/0F). Examination of the eyes of animals from the remaining ribavirin treatment groups revealed a low incidence of each lesion type, the incidence of which was not clearly dose related.

Comment: Increased retinal reflectivity in the rat may be indicative of retinal thinning and may correspond (as an indicator of retinal degeneration) with decreased retinal perfusion. While retinal degeneration is a fairly frequent sign of aging in the Sprague-Dawley rat, the incidence, onset and severity of the lesion appears to be increased by the chronic administration of ribavirin. This lesion, along with an increased frequency of microvascular hemorrhage, has been previously noted in 24 and 18 month carcinogenicity studies of ribavirin conducted in the rat and mouse, respectively.

Hematology and Clinical Chemistry: Similar to the effects noted after 25 weeks of ribavirin administration, at 50 or 52 weeks, ribavirin administration at 90 mg/kg, resulted in significant reductions (10-40%) in hemoglobin, hematocrit, erythrocyte counts and mean corpuscular hemoglobin concentration. These effects were generally evident throughout the dosing interval (beginning as early as week 4 of drug administration [earliest assessment]) and appeared to be non-progressive in extent. Similar, dose related, reductions in red cell parameters were evident among males and female animals dosed at 10 and 30 mg/kg/day.

Total white cell, lymphocyte, basophil and/or eosinophil counts were reduced (up to 50%) among surviving animals dosed at 90 mg/kg/day throughout the drug administration interval. Sporadic reductions in some white cell parameters were seen in animals treated with ribavirin at doses of 10-30 mg/kg. Despite these effects on circulating RBCs and WBCs, bone marrow smears taken at necropsy appeared normal with no consistent evidence of hypo- or compensatory hyper-cellularity.

Changes in serum chemistry noted at multiple time-points during the drug administration interval, included: a) reductions (10-20%) in serum ALT levels among animals dosed at 90 mg/kg/day (weeks 4-50), b) sporadic reductions in serum ALT levels among animals dosed at 30 mg/kg (♂: week 13; ♀: weeks 13 and 26), c) sporadic increases in serum AST levels among animals dosed at 90 mg/kg/day (week 4 only), and d) decreases (up to 25%) in serum protein and fluctuations in serum albumin levels (increases and decreases) among males and females dosed at $\geq$ 30 mg/kg/day. Among males, serum chloride levels were reduced slightly after 4 weeks of drug administration at levels of 10 and 30 mg/kg/day (but not at 90 mg/kg/day), while females dosed at 10-90 mg/kg/day for 26 weeks showed increases in serum phosphate levels (significant only among the high dose animals). Prothrombin time was not significantly altered by the administration of ribavirin at any dose tested. There were no other changes in any hematologic or serum chemistry parameter noted.

Gross and Microscopic Pathology: Similar to the effects noted after 25 weeks

of ribavirin administration, during the week 50-52 necropsy, increases in the absolute and/or relative weight of the heart and lungs were seen in both male and female animals treated with ribavirin at 90 mg/kg/day. Besides the increase in organ weight, the heart muscle of the affected animals appeared flaccid and dilated on gross examination. Thymus weights were decreased among the high and high-intermediate dose treated animals. Sporadic increases in the weight of the adrenals, spleen, kidneys and prostate were evident among ribavirin treated animals from all dose groups.

Microscopic examination of multiple lymphoid tissues revealed a generalized atrophy (thymus and lymph nodes) among male animals from the high-intermediate and high dose groups and among female animals from the high dose group. Lymphoid depletion was marked by significant loss of germinal centers, macrophage and other inflammatory cell infiltrates, and congestion. Extramedullary hemopoiesis was decreased or absent in the spleen of multiple animals dosed at 30 mg/kg/day and above. As at 25 weeks of ribavirin administration, the microscopic examination of tissues from the lungs of animals given ribavirin $\geq$ 30 mg/kg/day, showed an increased severity of alveolitis with macrophage infiltration and pneumonitis. Hyperplasia of the pulmonary arterioles, along with evidence of chronic myocarditis and myocardial fibrosis, was evident among some high dose male animals. A decreased incidence of basophilic foci was evident in liver sections from the high dose treated female animals.

Among the ribavirin treated male animals there was an increased incidence of swollen and/or bloated pituicytes in the anterior pituitary (sometimes called 'castration cells'). Interestingly, while an increase in abnormal pituitary cells was evident among the ribavirin dosed males, there was no microscopic evidence of an increase in the incidence of testicular degeneration among these animals. However, testicular degeneration, infertility and hypospermatogenesis have previously been noted in several species given ribavirin for $\geq$ 14 days (time-to-onset of effect varying between species); thus, the noted change in pituicyte morphology may reflect compensatory or antecedent effects to possible changes in testicular function/morphology.

The remaining gross and/or microscopic lesions appeared to be randomly distributed among the treatment and control groups.

Comments:

1) The study results clearly indicate that the administration of ribavirin was associated with a dose related suppression of multiple red blood cell parameters (HGB, HCT, MHC, etc.) and suppression of circulating white blood cells (particularly lymphocytes and eosinophils). These adverse effects were generally evident at the earliest toxicologic assessment, and continued throughout the dosing interval without significant progression or regression. Thrombocytosis was also evident among a subset of ribavirin treated animals.

2) The suppression of circulating white blood cells was accompanied by significant evidence of lymphoid tissue depletion and atrophy (loss of germinal centers, macro-phage and other inflammatory cell infiltrates, and congestion) of the thymus, spleen and lymph nodes among the affected animals. In contrast, the ribavirin induced suppression of multiple red blood cell parameters was not associated with any microscopic evidence of compensatory changes in the bone marrow.

3) As had been noted in several pre-GLP toxicologic studies of ribavirin, chronic exposure and/or high dose administration of ribavirin appears to be associated with significant histopathologic changes in the heart and lungs, and includes

significant signs of chronic inflammation, degeneration and fibrosis. Degenerative and vascular changes were also evident in the retinas of multiple high dose treated animals (male and female) as noted in the rat carcinogenicity of ribavirin.

4) The study results suggest that the concentration of ribavirin in plasma and erythrocytes may change slowly over a period of protracted drug exposure, with plasma levels gradually increasing while intra-erythrocyte drug levels decrease. However, these changes appear to be quite variable and modest in their extent.

5) The results of the present study suggest that ribavirin when given at doses $\geq$ 30 mg/kg/day, has significant adverse effects on rapidly proliferating tissues (particularly lymphoid tissues) and/or those tissues with the highest rate of cellular metabolism (heart and liver). These organs are likely to receive the highest levels of drug exposure following oral administration, due to the receipt of portal and central venous blood flow.

Cardiac and hepatic injuries have not been noted in the previously submitted GLP toxicology studies. Therefore, it is recommended that all oral repeat dose clinical trials with ribavirin include follow-up evaluations of cardiac and hepatic function.

6) As noted above, the administration of ribavirin was associated with reductions in serum proteins, reduc-tions in serum ALT levels, and sporadic increases in serum AST levels, among both male and female animals administered ribavirin at levels $\geq$ 30 mg/kg/day.

As discussed in relation to another study (conducted in the dog), no abnormalities were noted that might suggest a mechanism of increased protein/albumin loss, which therefore suggests that the changes in serum protein/albumin might be the result of decreased synthesis in the liver. Additional markers of hepatic function including serum CPK levels and mitochondrial citrate synthase or cytochrome oxidase were not measured.

DOG

5) **Ribavirin - 30 Day Pilot Oral (Gavage) Toxicity Study In The Beagle Dog,** Study No. 895/001.

Status: GLP
Study Site:
Study Initiation: 15 Jan. 1993
Compound Tested: Ribavirin, Lot# 05500787 (R-17),
Doses Tested: 0, 5, 10, 20 and 40 mg/kg/day
Dose Volume and Route: 5 ml/kg, gavage
Solvent: water for injection
Species, Strain, Sex: male & female Beagle dogs (Harlan), age 5 months, weight range: male = 7.4-11.2 kg; female = 6.3-9.2 kg, 3 animals/sex/dose.
Test conditions: Animals were randomly assigned to treatment groups. Ribavirin was administered by gavage once per day for a period of 30 days. Physical signs, behavior, mortality, body weight and food consumption were monitored regularly throughout the study. Hematologic analyses and clinical chemistries were performed on days -14, -7, 1, 14 and 29 of drug administration. ECG assessments were performed twice pretest, and on days 14 and 28 of drug administration. Gross and microscopic examinations of all standard tissues were performed at the termination of dosing.

Mortality: All animals from the high dose treatment group were sacrificed in extremis on dosing day 10, with clinical signs of extreme weight loss, inappetence, dark-loose-liquid stools with blood or mucus, prostration and vomiting. At euthanasia, these animals showed slight increases in hemoglobin concentration, red blood cell counts, hematocrit, total bilirubin, blood urea nitro-gen and heart rate. Other abnormalities noted, included a) hypo-plasia of the erythroid series of the bone marrow, and b) reduc-tions in serum electrolytes. The male animals appeared to be more severely affected by the drug treatment than were females at the same dose. There were no other premature deaths among the study animals.

Clinical Signs: As noted in the preceding section, an increased incidence of loose stools was evident among all of the high dose treated animals, but was also seen in several females from all of the drug treatment groups (i.e., 5-20 mg/kg/day) and in several males from the low and high-intermediate dose groups (i.e., 10 and 20 mg/kg/day). Post-dose vomiting was also noted among male and female animals from the low and high-intermediate dose groups (i.e., 10 and 20 mg/kg/day). There were no other clinical signs which appeared related to the administration of the test compound.

Body Weight and Food Consumption: As noted above, body weight was severely decreased (mean reduction of approximately 20%) within 1 week of the initiation of dosing, among male and female animals treated at 40 mg/kg/day. Several animals (2 male and 2 female) treated with ribavirin at 20 mg/kg/day displayed slightly reduced body weight or a reduction in weight gain when compared with the concurrent controls. In accordance with changes in body weight, food consumption was severely depressed among all of the high dose (40 mg/kg/day) treated animals, and was slightly depressed among the 4 (2 male and 2 female) high-intermediate dose (20 mg /kg/day) treated animals (which showed reduced terminal body weight). There were no other differences in body weight, weight gain or food consumption of animals in the control, low or low-intermediate dose groups.

Cardiac Examinations: At the time of death, several animals from the high dose treatment group (40 mg/kg/day) showed slight to moderate elevations in heart rate without significant changes in the P-R, QRS or R-T intervals. In addition, one female animal from the high-intermediate dose group displayed evidence of a second-degree atrio-ventricular block with extrasystole and bradycardia (mean heart rate of 50). No other abnormalities in ECG were noted among the drug treated or control animals.

Hematology and Clinical Chemistry: At the termination of drug treatment (Day 29), there was evidence of slight to moderate (5-15%) reductions in mean hemoglobin concentration, red blood cell counts and hematocrit among the high-intermediate dose (20 mg/kg /day) animals, and female animals from the low-intermediate dose (10 mg/kg/day) group. Lymphocyte counts were reduced (near 50%) among the high-intermediate dose treated male animals after 14 and 29 days of dosing. Among the high-intermediate dose treated animals, the bone marrow showed an increased proportion of erythroid cells. Similar, although somewhat smaller effects were evident following 14 days of drug administration. The effects noted among the female animals generally reached statistical significance ($p<0.05$), while those changes seen in the effected male animals were marginal or not statistically significant.

Changes in serum chemistry noted at the conclusion of dosing, included: a) decreases in serum protein (high-intermediate dose males and females, and low-intermediate dose treated females), b) reduced serum albumin (all drug treated female animals), c) a slight reduction in serum inorganic phosphorus levels (low- and high-intermediate dose groups) and potassium, and d) a slight dose related increase in serum creatinine.

No other changes in hematologic or serum chemistry parameters were noted as drug related among the treated animals.

Gross and Microscopic Pathology: At necropsy, increases in the absolute and/or relative weight of the adrenals was noted among male and female animals from the low-, high-intermediate and high dose groups, while decreases in both absolute and relative weight of the thymus was noted in these animals. The changes in thymic weight were generally dose related.

Microscopic examination of multiple tissues revealed significant evidence of lymphoid atrophy in the spleen, thymus, lymph nodes and Peyer's patches of the majority of high dose treated animals and in several high-intermediate dose treated animals. Further, among these animals there was evidence of suppression of the bone marrow, with the primary effect being on the erythroid cell series.

The remaining gross and/or microscopic lesions appeared to be randomly distributed among the treatment and control groups.

Comments: 1) As has been noted in several previously submitted toxicology studies, anemia, lymphopenia and thrombo-cytosis have been observed in nearly all animal species following repeat dosing with ribavirin. These effects may be associated with suppression of the bone marrow (as noted among the high dose treated dogs), and usually become evident within 4 weeks of the initiation of ribavirin administration. Ribavirin induced anemia has been of sufficient magnitude to require euthanasia of the test animals, or to require dose modification /cessation of drug administration.

2) As noted in the preceding sections, administration of ribavirin was associated with reductions in serum proteins and albumin among male animals dosed at 20 mg/kg/day (or higher), and among female animals at all drug levels tested (5-40 mg/kg/day). No histologic abnormalities were noted in the kidneys of the affected animals that might indicate a mechanism of increased protein/albumin loss (urine chemistry was not assessed, and serum creatinine remained within the normal range), which therefore suggests that the changes in serum protein/albumin might be the results of decreased synthesis. Thus, while serum transaminase levels remained within the normal range throughout treatment, the observed decreases in serum protein/albumin levels may have resulted from a ribavirin induced inhibition of liver synthetic capacity. (Unfortunately, several additional markers of hepatic function [including serum CPK levels, mitochondrial citrate synthase and cyto-chrome oxidase activity, and cellular triglyceride or cholesterol levels] were not measured.)

Obviously, potential hepatic dysfunction induced by a nucleoside analogue is of concern following the adverse sequelae of recent clinical trials with FIAU. However, it should be remembered that the proposed mechanisms of action for ribavirin are through incorporation into, or inhibition of the synthesis of RNA, with a resultant failure in the RNA message. Incorporation of ribavirin in the cellular genome has not been reported. Thus, if the DNA remains intact, and messenger RNA turnover is high, the removal of ribavirin from the system should result in recovery of any impaired functions.

3) The results of this study, suggest that 20 mg/kg/day likely represents the maximal survivable dose for a 12 month toxicity study in the beagle dog.

6) **Ribavirin: 52 Week Oral (Gavage) Toxicity Study In The Beagle Dog With An Interim Necropsy After 26 Weeks**, Study No. 895/002.

Status: GLP
Study Site:
Study Initiation: 23 March 1993
Compound Tested: Ribavirin, Lot# BR-17,
Doses Tested: 0, 5, 10 and 20 mg/kg/day
Dose Volume and Route: 5 ml/kg, gavage
Solvent: water for injection
Species, Strain, Sex: male & female Beagle dogs (Harlan), age 4 months, weight range: male = 6.1-8.9 kg; female = 5.0-8.2 kg, 8 animals/sex/dose (one-half of the study animals were terminated after 26 weeks of drug exposure as specified in the protocol).

Test conditions: Animals were randomly assigned to treatment groups. Ribavirin was administered by gavage once per day for a period of 52 weeks. Physical signs, behavior, mortality, body weight and food consumption were monitored regularly throughout the study. Hematologic analyses and clinical chemistries were performed pre-test, weekly for the first 6 weeks of dosing, and monthly thereafter. Ophthalmoscopic examinations were performed pre-test and once per month thereafter. ECG assessments were performed twice pretest, and after 5, 13, 25, 39 and 51 weeks of drug administration. Gross and microscopic examinations of all standard tissues were performed at the termination of dosing.

Results After 26 Weeks of Ribavirin Administration

Mortality: One male animal from the high dose treatment group was found dead during week 10 of drug administration following a brief period of decreased food consumption and weight loss. A post-mortem examination revealed significant fluid accumulation in the lungs, suggestive of a gavage accident. There were no other premature deaths among the study animals.

Clinical Signs: An increased incidence of loose stools was evident among several male and female animals from the high dose treatment group. Post-dose vomiting was also noted among male and female animals from the high dose group (i.e., 20 mg/kg /day). There were no other clinical signs which appeared related to the administration of the test compound.

Body Weight and Food Consumption: Body weight/weight gain were slightly decreased (mean reduction of about 20%) among male and female animals dosed at 20 mg/kg/day, adverse effects becoming evident within 1 week of the initiation of dosing. Males in the intermediate dose group also gained less weight during the period of drug treatment, although the mean absolute body weight of these animals was not statistically different from the control at the conclusion of dosing. Food consumption was reduced slightly (approx. 10%) among high dose treated animals, although the effect reached statistical significance only for the high dose treated females. There were no other differences in body weight, weight gain or food consumption of animals in the control, low or intermediate dose groups.

Cardiac Examinations: There were no obvious drug related effects on any ECG parameter noted among the drug treated animals.

Ophthalmoscopy: There were no obvious drug related effects, although one animal in the high dose group and another from the intermediate dose group developed corneal or lens associated opacities within 8-13 weeks of the start of drug administration.

Hematology and Clinical Chemistry: After 2-8 weeks of drug administration, there was evidence of slight to moderate (5-30%) reductions in mean hemoglobin concentration, RBC counts, hemato-crit, mean corpuscular hemoglobin and mean